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PATENT
Attorney Docket No. 400684/SOEI

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RECEIVED

In re Application of:

JUN 04 2002

ISHIBASHI et al.

TECH CENTER 1600/2900

Application No. 09/775,818

Art Unit: 1655

Filed: February 5, 2001

Examiner: Ethan Whisenant

For: METHOD FOR SELECTIVELY
SEPARATING LIVE CELLS
EXPRESSING A SPECIFIC GENE

RESPONSE

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In response to the Office Action dated January 30, 2002, please enter the following amendments and consider the following remarks. A page showing the requested change is attached. This response is filed timely as it is accompanied by a petition and fee for a one-month extension for filing a response under 37 C.F.R. § 1.136(a).

AMENDMENTS

IN THE CLAIMS:

Replace the indicated claim with:

B¹
4. (Amended) The method according to claim 2, wherein the selective separation in said third step of said live cells based on the change in fluorescence is performed by a cell sorter.

⌈ Add the following claim: ⌋

10. (New) A method for selectively separating live cells which have expressed mRNA comprising:

B²
a first step of introducing a marker capable of labeling mRNA into cells in a live cell group containing live cells which have expressed a specific mRNA by a method selected from the group consisting of microinjection method, electroporation method and lipofection method;

In re Appln. of ISHIBASHI et al.
Application No. 09/775,818

a second step of labeling said mRNA with said marker to obtain a live cell group containing live cells having the labeled mRNA; and

352 a third step of detecting said labeled mRNA to identify the live cells having said labeled mRNA and separating the identified live cells selectively from said live cell group obtained in said second step.

REMARKS

Discussion of Claim Amendments

Applicants have deleted "(FACS)" in claim 4, as the deleted term is unnecessary. New claim 10 has been added and is directed to an embodiment of the invention. The amended and new claims are fully supported by the original claims and the specification, e.g., at page 45, lines 20-24. No new matter has been added.

The Office Action

The Office Action sets forth the following grounds for rejection: (1) claim 4 is rejected under 35 U.S.C. § 112, second paragraph, for an alleged indefiniteness; (2) claims 1-2 and 4 are rejected under 35 U.S.C. § 102(b), as allegedly anticipated by U.S. Patent 5,629,147 (Asgari et al.); and (3) claims 3, 5, 7, and 9 are rejected under 35 U.S.C. § 103(a), as allegedly unpatentable over Asgari et al. and U.S. Patent 6,228,592 (Tsuji et al.).

The Present Invention

The present invention relates to a method for selectively separating live cells which have expressed a specific gene. Claims 1-10 are currently pending. A set of pending claims is attached.

Discussion of Indefiniteness Rejection

Applicants have amended claim 4 as discussed above. The amended claim renders the rejection moot. In view of the foregoing, the indefiniteness rejection of claim 4 should be withdrawn.

Discussion of Anticipation Rejection

The Office Action states that Asgari et al. teaches a method for selectively separating live cells expressing a particular mRNA from cells which do not express said particular mRNA comprising the three steps recited in present claim 1. The Office Action refers to Examples 22-28 of Asgari et al. The Office Action further states that Asgari et al. teaches all of the limitations recited in present claims 2 and 4. Applicants respectfully traverse this rejection.

Asgari et al. fails to disclose a method which uses live cells. The methods disclosed in Asgari et al. include a step of hybridizing a fluorescently labeled oligonucleotide probe with mRNA in a cell and a step of identifying the hybridized mRNA. The methods disclosed in Asgari et al. include in situ hybridization.

In situ hybridization requires, those of skill in the art would know, the process of fixation of cells and/or partial destruction of cell membranes prior to hybridization of the

probe to the target mRNA. The fixation of cells is performed by treatment of the cells with ethanol, acetic acid, methanol, acetone, or solutions containing paraformaldehyde, glutaraldehyde, or formaldehyde. Partial destruction (pore-formation) of cell membranes is performed by treatment of the fixed cells with solutions containing a detergent such as Brij 35, Brij 58, sodium dodecyl sulfate, CHAPS (column 20, line 16), or Triton X-100. Detailed descriptions of the fixation and pore-formation processes can be found in columns 16-17 of Asgari et al. Particularly, fluorescently labeled oligonucleotides are hybridized with target mRNA in the cells which are no longer alive due the fixation and pore-forming treatments. The above treatments of live cells result in death of the cells.

In Asgari et al. Examples 23-27, cells are treated with 0.5% paraformaldehyde overnight. See columns 16-17 (Hybridization Fixative, Hybridization Solutions Components). In Examples 22 and 23, in situ hybridization was performed after cells were separated by a cell sorter. In Examples 24-28, the cells (dead cells) upon which in situ hybridization was performed were separated by a cell sorter. As can be seen in the following explanations of Examples 22-28, all the examples include in situ hybridization that kills live cells. Therefore, Examples 22-28 are different from the invention recited in claim 1 of the present invention. A short summary of Asgari et al. Examples 22-28 is provided below.

<Example 22>

Cells were introduced into a flow cytometer (cell sorter) and cell separation was carried out on the basis of the strengths of forward scattering light and side scattering light. The separated cells were deposited on the slides. In situ hybridization was performed on HbF (fetal hemoglobin) mRNA, the X chromosome and the Y chromosome in order to confirm the existence of fetal cells. See column 29, lines 32-62 for a detection method involving in situ hybridization of HbF mRNA and column 24, line 1 to column 25, line 8 for a detection method involving in situ hybridization of the X and Y chromosomes.

<Example 23>

Cells were labeled with anti-CD45 antibodies labeled with FITC (a fluorescent dye). The cells were also treated with Hoechst dye (a dye that stains nucleus). The treated cells were introduced into a flow cytometer to separate cells on the basis of 3 characteristics of (1) the fluorescence of Hoechst dye, (2) the strengths of forward scattering light and side scattering light and (3) the fluorescence of FITC. The separated cells were deposited on the slides. In situ hybridization was performed on HbF mRNA, the X chromosome and the Y chromosome in order to confirm the existence of fetal cells.

<Example 24>

Cells were fixed by treatment with 0.5% paraformaldehyde overnight. On the next day, in situ hybridization was carried out on HbF mRNA. The resultant cells were introduced into a flow cytometer to separate cells on the basis of 3 characteristics: (1) the fluorescence of Hoechst dye, (2) the strengths of forward scattering light and side scattering light and (3) the fluorescence of fluorescent probe for HbF. The separated cells were deposited on the slides. Signals from HbF mRNA, the X chromosome and the Y chromosome were determined in order to confirm the existence of fetal cells.

<Example 25>

Cells were treated with anti-CD45 antibodies labeled with PE (a fluorescent dye). The treated cells were fixed by treatment with 0.5% paraformaldehyde overnight. On the next day, in situ hybridization was carried out on HbF mRNA. The resultant cells were introduced into a flow cytometer to separate cells on the basis of 4 characteristics: (1) the fluorescence of Hoechst dye, (2) the strength of forward scattering light and side scattering light, (3) the fluorescence of fluorescent probe for HbF, and (4) the fluorescence of PE. The separated cells were deposited on the slides. Signals from HbF mRNA, the X chromosome and the Y chromosome were determined in order to confirm an existence of fetal cells.

<Example 26>

Cells were treated with anti-CD45 antibodies labeled with PE. The treated cells were fixed by treatment with 0.5% paraformaldehyde overnight. On the next day, in situ hybridization was carried out on HbF mRNA and HbA (maternal hemoglobin) mRNA. The resultant cells were introduced into a flow cytometer to separate cells on the basis of 5 characteristics: (1) the fluorescence of Hoechst dye, (2) the strengths of forward scattering light and side scattering light, (3) the fluorescence of fluorescent probe for HbF, (4) the fluorescence of fluorescent probe for HbA and (5) the fluorescence of PE. The separated cells were deposited on the slides. Signals from HbF mRNA, the X chromosome and the Y chromosome were determined in order to confirm the existence of the fetal cell.

<Example 27>

Cells were treated with anti-CD45 antibodies labeled with PE. The treated cells were fixed by treatment with 0.5% paraformaldehyde overnight. On the next day, in situ hybridization was carried out on HbA mRNA. The resultant cells were introduced into a flow cytometer to separate cells on the basis of 4 characteristics: (1) the fluorescence of

Hoechst dye, (2) the strengths of forward scattering light and side scattering light, (3) the fluorescence of fluorescent probe for HbA and (4) the fluorescence of PE. The separated cells were deposited on the slides. Signals from HbF mRNA, the X chromosome and the Y chromosome were determined in order to confirm the existence of fetal cells.

<Example 28>

Cells were labeled with anti-CD45 antibodies labeled with FITC (a fluorescent dye). The cells were also treated with Hoechst dye (a dye that stains nucleus). The treated cells were introduced into flow cytometer to separate cells on the basis of 3 characteristics: (1) the fluorescence of Hoechst dye, (2) the strengths of forward scattering light and side scattering light and (3) the fluorescence of FITC. The separated cells were deposited on the slides. In situ hybridization was performed on Trophoblast mRNA, the X chromosome and the Y chromosome in order to confirm the existence of fetal cells.

In view of the foregoing, Asgari et al. does not disclose the invention recited in claim 1. Claim 2 and 4 are directly or ultimately dependent upon claim 1. Therefore, the anticipation rejection of claim 1, 2, and 4 should be withdrawn. Claim 10 also should not be rejected on this basis.

Discussion of Obviousness Rejection

Claims 3, 5, 7, and 9 are rejected under 35 U.S.C. § 103(a), as allegedly unpatentable over Asgari et al. in view of Tsuji et al. Applicants respectfully traverse this rejection.

The subject matter of Tsuji et al. was subjected to an assignment to Laboratory of Molecular Biophotonics at the time the invention was made. The present application was originally assigned to the same assignee, Laboratory of Molecular Biophotonics. See attached copies of the Notice of Recordation of Assignment in Tsuji et al. and the present application. Both Tsuji et al. and the present application are now assigned to Hamamatsu Photonics K.K. 35 U.S.C. § 103(c) applies. In view of the foregoing, the obviousness rejection of claims 3, 5, 7, and 9 should be withdrawn. Claim 10 also should not be rejected on this basis.

The European patent application that corresponds to Tsuji et al. was laid-open on November 15, 2000 as EP 1052293 (Application No. 99 129 030) (copy attached). However, the present application claims priority to JP2000-028117 filed on February 4, 2000 and JP2000-130793 filed on April 28, 2000. Certified English language translations of the priority applications are enclosed. In view of the foregoing, the present claims should not be rejected over EP 1052293.

In re Appln. of ISHIBASHI et al.
Application No. 09/775,818

Conclusion

The application is considered in good and proper form for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,



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**AMENDMENT TO CLAIM MADE IN RESPONSE
TO OFFICE ACTION DATED JANUARY 30, 2002**

Amendments to existing claim:

4. (Amended) The method according to claim 2, wherein the selective separation in said third step of said live cells based on the change in fluorescence is performed by a cell sorter (~~FACS~~).



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**PENDING CLAIMS AFTER AMENDMENTS
MADE IN RESPONSE TO OFFICE ACTION DATED JANUARY 30, 2002**

1. A method for selectively separating live cells which have expressed mRNA comprising:
 - a first step of introducing a marker capable of labeling mRNA into cells in a live cell group containing live cells which have expressed a specific mRNA;
 - a second step of labeling said mRNA with said marker to obtain a live cell group containing live cells having the labeled mRNA; and
 - a third step of detecting said labeled mRNA to identify the live cells having said labeled mRNA and separating the identified live cells selectively from said live cell group obtained in said second step.
2. The method according to claim 1, wherein said marker in said first step is a probe which has a base sequence complementary to said mRNA and has been labeled with a fluorescent dye, said labeled mRNA in said second step is a hybrid of the probe and said mRNA, and the selective separation in said third step is performed by irradiating light to the live cell group containing live cells having the hybrid, identifying live cells which cause a change in fluorescence of said fluorescent dye based on formation of the hybrid, and separating the identified live cells from the live cell group.
3. The method according to claim 2, wherein said probe comprises a first probe and a second probe, the first probe and the second probe have base sequences capable of hybridizing to said mRNA adjacently, the first probe is labeled with an energy donor fluorescent dye and the second probe is labeled with an energy acceptor fluorescent dye, and said change in fluorescence is caused by fluorescence resonance energy transfer (FRET) from the energy

donor fluorescent dye of the first probe to the energy acceptor fluorescent dye of the second probe.

4. The method according to claim 2, wherein the selective separation in said third step of said live cells based on the change in fluorescence is performed by a cell sorter.

5. The method according to claim 1, wherein said mRNA is an mRNA encoding a cytokine.

6. The method according to claim 3, wherein said mRNA is an mRNA encoding interleukin-2 (IL-2), said first probe is a probe having a base sequence set forth in SEQ ID NO: 9 in the Sequence Listing, and said second probe is a probe having a base sequence set forth in SEQ ID NO: 10 in the Sequence Listing.

7. The method according to claim 1, wherein the live cells selectively separated in said third step are T Helper 1 (TH1) cells.

8. The method according to claim 3, wherein said mRNA is an mRNA encoding interleukin-4 (IL-4), said first probe is a probe having a base sequence set forth in SEQ ID NO: 17 in the Sequence Listing, and said second probe is a probe having a base sequence set forth in SEQ ID NO: 18 in the Sequence Listing.

9. The method according to claim 1, wherein the live cells selectively separated in said third step are T Helper 2 (TH2) cells.

10. A method for selectively separating live cells which have expressed mRNA comprising:
a first step of introducing a marker capable of labeling mRNA into cells in a live cell group containing live cells which have expressed a specific mRNA by a method selected from the group consisting of microinjection method, electroporation method and lipofection method;

a second step of labeling said mRNA with said marker to obtain a live cell group containing live cells having the labeled mRNA; and

a third step of detecting said labeled mRNA to identify the live cells having said labeled mRNA and separating the identified live cells selectively from said live cell group obtained in said second step.